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ACUTE DERMAL IRRITATION STUDY OF SIX JET FUELS IN NEW ZEALAND WHITE RABBITS: COMPARISON OF FOUR BIO-BASED JET FUELS WITH TWO PETROLEUM JP-8 FUELS

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The experiments reported were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

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14. ABSTRACT
The objective of this study was to determine the irritative potential of two petroleum derived operational jet fuels and four alternative fuels following a single exposure to the skin of New Zealand White rabbits. Operational fuels included two JP-8 formulations: JP-8 containing 21.25 mass percent aromatic components as a baseline control for the study and a second high aromatic (HA) JP-8 containing 28.05 mass percent aromatics for comparison with higher aromatic alternative fuels. The alternative fuels assessed were KiOR hydroprocessed depolymerized cellulosic jet (HDCJ) fuel; ReadiJet fuel produced by Applied Research Associates; Amyris C15 Direct Sugar to Hydrocarbon (DSHC); and Virent Synthetic Aromatic Kerosene (SAK). There were no significant differences in irritation noted between the baseline fuels (JP-8 and HA JP-8) and the four alternate jet fuels; all were slightly irritating when exposed under occluded and semi-occluded conditions, with the exception of "ReadiJet" which was nonirritating when exposed semi-occluded. It is not expected that normal handling of these fuels would result in increased dermal irritation among airmen.

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PREFACE

Funding for this project was provided through the Defense Logistics Agency (DLA)/Energy. The program manager for DLA was Yan Guo. This research was conducted under contract FA8650-10-2-6062 with the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF). The program manager for the HJF contract was David R. Mattie, PhD (711 HPW/RHDJ), who was also the technical manager for this project.

The dermal irritation study protocol was designed to be in general compliance with the U.S. Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870.2500 (U.S. EPA, 1998) and the Organisation for Economic Co-operation and Development Guidelines (OECD) for Testing of Chemicals, Section 404 (2002). The study was conducted in compliance with 40 CFR Part 792, Good Laboratory Practice Standards (GLP).

The study protocol was approved by the Air Force Surgeon General's Office of Research Oversight and Compliance (protocol number FWR-2013-006A, Acute Dermal Irritation Study of Four Alternative Jet Fuels Plus Two Baseline JP-8 Fuels in New Zealand White Rabbits) and the WIL Research Laboratories, LLC, Animal Care and Use Committee (protocol number WIL-773004). The study was conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011).

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1.0 SUMMARY

The objective of this study was to determine the irritative potential of four alternative fuels and compare them to two petroleum derived operational jet fuels following a single exposure to the skin of New Zealand White rabbits. Each fuel was tested under occluded and semi-occluded conditions. The protocol was designed to be in general compliance with the U.S. Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870.2500 (U.S. EPA, 1998), the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 404 (2002), and the European Union Guideline in the Official Journal of the European Communities [92/69, Annex V, B4 (1992)].

All fuels were identified by a commercial name and by the POSF log book number provided by the Air Force Research Laboratory Fuels Branch (AFRL/RQTF, Wright-Patterson AFB OH). All fuels contained the standard JP-8 additive package. Use of commercial names and products does not constitute endorsement of these products by the U.S. Air Force but does reflect the variety of jet fuels being tested for performance and, therefore, the breadth of fuels to which airmen may be exposed, now or in the future.

Operational fuels included two JP-8 formulations, the first a previously examined JP-8 (POSF 4658) containing 21.25 mass percent aromatic components as a baseline control for the study. The second JP-8 (POSF 8457) contained 25 volume percent/28.05 mass percent aromatics and served as a high aromatic (HA JP-8) baseline for comparison with higher aromatic alternative fuels.

The alternative fuels assessed were:

- KiOR hydroprocessed depolymerized cellulosic jet (HDCJ) fuel (POSF 9818) made by KiOR, Inc. (Pasadena TX);
- ReadiJet (Renewable, Aromatic, Drop-in, abbreviated Readi) fuel (POSF 10136) produced by Applied Research Associates (ARA, Albuquerque NM);
- Amyris C15 direct sugar to hydrocarbon (DSHC, more than 97 mass percent farnesane, POSF 10150) produced by Amyris Biotechnologies (Emeryville CA); and
- Virent synthetic aromatic kerosene (SAK, 10326) produced by Virent, Inc. (Madison WI).

There were no significant differences in irritation noted between the baseline fuels (JP-8 and HA JP-8) and the four alternate jet fuels when administered dermally. All were slightly irritating when exposed occluded and semi-occluded with the exception of “ReadiJet” which was nonirritating when exposed semi-occluded. It is not expected that normal handling of these fuels would result in increased dermal irritation among airmen.

2.0 INTRODUCTION

In order to reduce dependence on foreign crude oil, domestically produced alternative fuels are being pursued for military use (Blackwell, 2007). These alternative fuels are formulated to be used as drop-in-technology, in combination with or in place of petroleum-derived JP-8, the single battlefield fuel of the U.S. Air Force. Each alternative fuel differs compositionally from petroleum-derived JP-8; therefore, the potential for toxicity from each fuel must be assessed.

The purpose of this report was to investigate six jet fuels for dermal irritation potential (see Table 1 for fuel descriptions). All fuels were identified not only by a commercial name but also by the POSF log book number maintained by the Air Force Research Laboratory (AFRL) Fuels and Energy Branch (AFRL/RQTF, Wright-Patterson AFB OH). Use of commercial names and products does not constitute endorsement of these products by the U.S. Air Force but does reflect the variety of jet fuels to which airmen may be exposed, now or in the future. All fuels contained the standard JP-8 additive package required by the U.S. Air Force.

Table 1. Test Substance Identification and Description

Test Substance	Identification & Definition	POSF	POSF (+ additives)	WIL ID #	Physical Description
1	**JP-8** Baseline fuel	4658	4658+	13027E	Clear, light yellow liquid
2	**HA JP-8** High Aromatic JP-8	8457	8457+	13027F	Clear, light yellow liquid
3	**KiOR HDCJ** Hydroprocessed Depolymerized Cellulose	9818	10327	130280	Clear, light yellow liquid
4	**ReadiJet**	10136	10328	130281	Clear, light yellow liquid
5	**Amyris C15 DSHC** Direct Sugar to Hydrocarbon	10150	10329	130282	Clear, colorless liquid
6	**Virent SAK** Synthetic Aromatics, Kerosene range	10326	10330	130283	Clear, light yellow liquid

Notes: POSF: AFRL Fuels and Energy Branch log book numbers - Fuels are assigned different POSF numbers following addition of JP-8 additive package; WIL ID #: identification numbers maintained by WIL Research Laboratories, LLC

Two operational fuels provided points of comparison for the alternative fuels tested in this study. The first fuel utilized was petroleum-derived JP-8 (POSF 4658); the dermal irritancy of this fuel has been assessed previously and has been included as a baseline "positive" control for response comparison when alternative fuels are tested (Hurley *et al.*, 2011; Mattie *et al.*, 2013). An additional petroleum JP-8 with a higher aromatic content was also evaluated in order to provide a

point of comparison for the higher aromatic content of some of the alternative fuels; this fuel has been abbreviated as HA JP-8 throughout the report. The specification maximum for aromatics in JP-8 is 25 percent by volume (DoD, 2013). The HA JP-8 contains 25 volume percent aromatics; comprehensive two-dimensional gas chromatography (GC x GC) has identified the fuel to contain 28.05 mass percent aromatics. The baseline JP-8 contains 21.25 mass percent aromatic compounds. A summary of the GC x GC analysis of the fuels is provided in Table 2. A more comprehensive analysis of the fuels can be found in Appendix A.

Table 2. Summary GC x GC Component Comparison of Test Substances

Fuel	JP-8	High Aromatic JP-8 (HA JP-8)	KiOR HDCJ	ReadiJet	Amyris C15 DSHC (farnesane)	Virent SAK
POSF*	4658	8457	9818	10136	10198, 10324, 10323**	10326
AROMATICS						
Total Alkylbenzenes	13.69	20.41	12.10	10.35	0.10	92.82
Total Alkylnaphthalenes	1.76	3.26	1.36	0.52	<0.01	0.03
Total Cycloaromatics	5.79	4.38	39.55	7.69	<0.01	4.53
Total Aromatics	**21.25**	**28.05**	**53.02**	**18.56**	**0.10**	**97.38**
ALIPHATICS						
Total iso-Paraffins	31.34	29.21	0.61	8.63	97.90	0.72
Total n-Paraffins	19.00	19.46	0.12	30.18	<0.01	0.39
Total Cycloparaffins	28.42	23.29	46.25	42.63	1.00	1.51
Total Aliphatics	**78.75**	**71.95**	**46.98**	**81.44**	**98.90**	**2.62**
Total Alcohols	<0.1	<0.1	<0.1	<0.1	**1.00**	<0.1
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Note: Component values given in mass percent. *Component analysis is performed on the fuel prior to addition of additives. **The batches of Amyris fuels, denoted by different POSF numbers, are analogous to the POSF 10150 fuel used in the dermal study; the Amyris process provides very similar batches of product that are approximately 97 mass percent farnesane. POSF: AFRL Fuels and Energy Branch log book numbers.

Four alternative jet fuels formulated from different processes and various feedstocks were evaluated for dermal irritation potential. All four of these fuels are also being evaluated for U.S. Air Force suitability at the AFRL Research Laboratory. The first is a hydroprocessed depolymerized cellulosic jet (HDCJ) fuel made by a company called KiOR, Inc. (Pasadena TX). This pyrolysis-based fuel is made from wood, primarily southern yellow pine, using a proprietary catalyst system known as Biomass Fluid Catalytic Cracking, followed by conventional hydrotreatment. Building of a Columbus MS plant capable of producing 11 million

gallons per year of this fuel began in early 2013 (KiOR, 2014). This fuel is roughly 50 percent aromatics and 50 percent cycloparaffins by mass (Table 2); it is expected to be blended at 30 percent by volume with a baseline JP-8 (not HA JP-8) or, sometime in the future, at a higher percentage with very low aromatic (less than 1 mass percent) alternative fuels such as hydroprocessed esters and fatty acids-synthetic paraffinic kerosene (HEFA-SPK) or Fischer-Tropsch (FT)-SPK. The 30 percent/70 percent volume/volume blend with baseline JP-8 was successfully engine tested for the Federal Aviation Administration (FAA) Continuous Lower Emissions, Energy and Noise (CLEEN) program in 2013 (Pratt and Whitney, 2013).

The second alternative fuel, ReadiJet, refers to a Renewable, Aromatic, Drop-in (abbreviated Readi) fuel produced from plant oils or animal fats through a catalytic hydrothermolysis process to convert the triglycerides to an aromatic-containing fuel. Produced by Applied Research Associates (ARA, Albuquerque NM), the product is suitable for use at 100 percent as a fully synthetic/renewable fuel (ARA, 2014). ReadiJet has already been flown at 100 percent in a Canadian commercial aircraft in 2012 (GreenAirOnline.com, 2013). ARA and the AFRL Fuels and Energy Branch have collaborated in producing a target 15 volume percent aromatic version of ReadiJet (18 mass percent, Table 2) for performance and toxicity testing. This ReadiJet batch successfully completed fit-for-purpose testing in the FAA CLEEN program, also in 2013 (Pratt and Whitney, 2013).

A third alternative fuel, Amyris C15 direct sugar to hydrocarbon (DSHC), is very different from the other fuels. Produced from fermentation of sugar by engineered microorganisms, this fuel is more than 97 percent farnesane (trimethyl dodecane, a 15-carbon isoparaffin, CAS number 3891-98-3) by mass. This fuel is produced by Amyris Biotechnologies (Emeryville CA), which has a commercial scale plant in Brazil utilizing sugarcane as a feedstock (Amyris, 2014). Due to the low freezing point inherent for an iso-paraffin, 10 to 20 percent (by volume) farnesane mixed with JP-8 is the proposed mixture for use by the Air Force. In Europe, farnesane is sold as a diesel fuel, so some toxicity testing on the neat material (approximately 98 percent pure) has been completed. Tests include dermal irritation, in which farnesane was found to be the equivalent of slightly irritating using a comparable assay in rabbits. Farnesane was judged non-irritating in two *in vitro* assays: the MatTek™ Epiocular MTT viability eye irritation assay and MatTeck™ Epiderm skin irritation assay. Human 48-hour patch tests indicated that farnesane is a very mild irritant. Human repeat insult patch testing (HRIPT) revealed that farnesane is only irritating under localized, occluded conditions; dilutions (60 percent or less) or un-occluded patches of neat material resulted in no irritation (Amyris, 2012).

The final alternative fuel, Virent synthetic aromatic kerosene (SAK), is produced by Virent, Inc. (Madison WI) by combining aqueous phase reforming (APR) technology and modified catalytic conversion of sugars, including cellulosic sugars, to produce fuel range hydrocarbons (Virent, 2014). SAK is primarily composed of aromatic constituents (Table 2). The SAK fuel is currently undergoing fit-for-purpose testing at Southwest Research Institute (San Antonio TX).

The objective of the following study is to determine dermal irritation potential of these higher aromatic alternative fuels. The results will be compared directly with conventional (baseline) JP-8 and a higher aromatic content petroleum JP-8 (HA JP-8). The results will also be compared with dermal irritation studies previously conducted for petroleum based and alternative jet fuels.

3.0 METHODS

The complete study protocol, including an amendment and a deviation, is found in Appendix B.

3.1 Test Substances Identification

Test substances were received from the AFRL Fuels and Energy Branch (AFRL/RQTF), Wright-Patterson Air Force Base (AFB) OH, on 6 Aug 2013. Test substances are identified by POSF log book numbers as well as WIL identification numbers (Table 1). Purity and stability data are maintained by the AFRL Fuels and Energy Branch.

The test substances were stored at room temperature in a flame cabinet and were considered stable under these conditions. Prior to use, the original container of each test substance was inverted or swirled to ensure a homogeneous mixture. A reserve sample of each test substance was collected and stored in the WIL Archives.

3.2 Animals and Handling

Male New Zealand White albino rabbits were used as the test system in this study; this animal model is generally recognized as appropriate for acute dermal irritation studies. The number of animals selected was the minimum required to satisfy regulatory guidelines. The experimental design used procedures and standards required by the current federal and international test guidelines. The animals were young adults (approximately 7 months of age) at the initiation of dose administration.

Male New Zealand White albino rabbits utilized for this study were received in good health from Covance Research Products, Inc. (Denver PA). The rabbits were inspected by a qualified technician upon receipt, weighed and uniquely identified by a subcutaneous BMDS microchip (BioMedic Data Systems, Inc., Seaford DE) implanted in the dorso-scapular area. The rabbits were acclimated to laboratory conditions for a minimum of five days. During this period, each animal was observed twice daily for changes in general appearance or behavior.

Animals were housed in individual stainless steel cages. The animals were maintained by the animal husbandry staff of WIL in accordance with standard operating procedures (SOPs). The animal facilities at WIL are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Devices were provided to all animals as appropriate for environmental enrichment.

The basal diet used in this study, PMI Nutrition International, LLC (St. Louis MO) Certified Rabbit HF LabDiet® 5325, is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL. Municipal water supplying the facility was analyzed for contaminants according to WIL SOPs. The results of the diet and water analyses are maintained at WIL. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study. The basal diet was provided at approximately 150

g/day while municipal water was provided *ad libitum* by an automatic watering system throughout the acclimation period and during the study.

All animals were housed throughout the acclimation period and during the study in an environmentally controlled room. The room temperature and humidity controls were set to maintain environmental conditions of 66 ± 5°F (19 ± 3°C) and 50 ± 20 percent relative humidity. Room temperature and relative humidity data were monitored continuously and recorded automatically on an hourly basis. Illumination was provided for a 12 hour light (0600 hours to 1800 hours) and 12 hour dark photoperiod using fluorescent lighting. Air handling units were set to provide a minimum of 10 fresh air changes per hour.

3.3 Administration

This study was intended to provide information on the health hazards likely to arise from a short-term exposure to the test substances by the dermal route. The selected route of administration for this study was direct application to clipped, unabraded skin (dermal), standard for assessment of local dermal irritative potential. The experimental design used the procedures and standards required by the current federal and international regulations. In this assay, each rabbit serves as its own control as there are up to six sites per rabbit on which to apply individual fuels and plenty of untreated skin to serve as the control. Dermal irritation assays are performed using two sets of three rabbits; the application sites are occluded in one set and semi-occluded in the second set. Table 3 presents the study group assignment.

Table 3. Study Group Assignment

Group Number	Test Substance	Dose Volume (mL)	Exposure Method	Number of Animals
1	1 through 6	0.5	Occluded	3
2	1 through 6	0.5	Semi-occluded	3

Note: Test substances are identified in Table 1.

On the day prior to dosing, the hair was removed from the backs and flanks of the rabbits using an electric clipper. Six application sites were available for dosing, as depicted in Figure 1. Fuels were assigned a site in rotation (first fuel applied to site A on first rabbit, B on second rabbit, etc.).

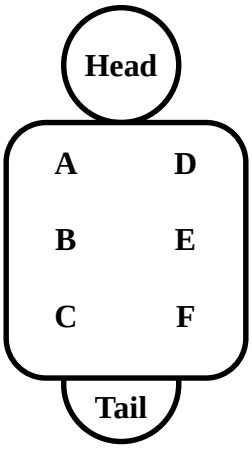

Figure 1. Dermal Dosing Sites on New Zealand White Rabbits

Each 0.5-mL dose was applied to an area of skin approximately 2.5 cm × 2.5 cm under a two-ply gauze patch secured in place with Micropore™ tape (3M, St. Paul MN). For animals in the occluded exposure groups, the trunk of the animal was wrapped with plastic wrap to occlude the test site. The trunk of animals in both the occluded and semi-occluded groups was then wrapped with a gauze binder secured with Dermiform® tape (Johnson and Johnson, New Brunswick NJ). Plastic restraint collars were applied to the animals to prevent ingestion of the test substance and/or bandages. After four hours of exposure, the collars and bandages were removed and each of the sites was wiped with a new disposable paper towel moistened with deionized water.

3.4 Observations

The rabbits were observed twice daily, once in the morning and once in the afternoon, for mortality and morbidity. All animals received detailed physical examinations on the day of dosing. Body weights were obtained and recorded on study day 0 (initiation) and at each rabbit's termination from the study.

The application sites were observed for erythema, edema and other dermal findings approximately 30 to 60 minutes and 24, 48 and 72 hours after patch removal, and again on study days 4, 7 and 14. Dermal irritation was graded in accordance with the method of Draize (1965), as detailed in Appendix C. The areas of application were clipped free of hair a minimum of one hour prior to scoring, as needed during the study, to facilitate accurate dermal observations.

The Primary Dermal Irritation Index (PDII) was calculated from scores recorded at 30 to 60 minutes and at 24, 48 and 72 hours after patch removal. The mean scores for erythema and edema were calculated separately to the nearest tenth and added together. Based on this value, the grading system in Appendix C was used to arrive at the PDII descriptive rating.

All data were recorded and reported utilizing WIL in-house data systems detailed in Appendix D. Following study termination, the rabbits were euthanized in accordance with current American Veterinary Medical Association (AVMA) guidelines (AVMA, 2013).

4.0 RESULTS

4.1 General Conditions and Observations

Animal room conditions are summarized in Appendix E. Actual mean daily temperature ranged from 65.3°F to 65.9°F (18.5°C to 18.8°C) and mean daily relative humidity ranged from 43.7 to 52.6 percent during the study.

There were no deaths during the study. Body weight values ranged from 2954 g to 3273 g at the initiation of dosing. No test substance-related body weight changes were noted during the study. Individual body weights are detailed in Table 4.

Table 4. Individual Body Weights

Group Number	Test Substance	Animal Number	Day 0 Weight (g)	Day 15 Termination Weight (g)
1 Occluded	1 through 6	2964	3036.8	3196.4
		2965	3272.9	3380.2
		2966	3106.7	3284.9
2 Semi-Occluded	1 through 6	2967	3097.8	3235.8
		2968	3232.4	3340.0
		2969	2954.4	3031.7

4.2 Dermal Observations

There were no significant differences in irritation noted between the baseline fuels (JP-8 and HA JP-8) and the four alternate jet fuels when administered dermally. Overall, the fuels were slightly irritating when exposed occluded and semi-occluded with the exception of "ReadiJet" which was nonirritating when applied under semi-occluded conditions. These results are summarized in Table 5. Individual score data are found in Appendix F. Application site assignments for each fuel/rabbit are also found in Appendix F.

Table 5. Summary Table of PDII and Descriptive Rating for Dermal Irritation

Test Substance	Identification	Exposure	PDII	Descriptive Rating
1	JP-8	Occluded	0.6	Slightly Irritating
		Semi-occluded	0.3	Slightly Irritating
2	HA JP-8	Occluded	0.2	Slightly Irritating
		Semi-occluded	0.2	Slightly Irritating
3	KiOR HDCJ	Occluded	0.4	Slightly Irritating
		Semi-occluded	0.3	Slightly Irritating
4	ReadiJet	Occluded	0.3	Slightly Irritating
		Semi-occluded	0.0	Nonirritating
5	Amyris C15 (farnesane) DSHC	Occluded	0.7	Slightly Irritating
		Semi-occluded	0.2	Slightly Irritating
6	Virent SAK	Occluded	0.7	Slightly Irritating
		Semi-occluded	1.5	Slightly Irritating

Specifically, the baseline fuels (JP-8 and HA JP-8) resulted in the descriptive rating of slightly irritating, with the HA JP-8 having lower PDII scores than the baseline JP-8 formulation (Table 5). JP-8 occluded exposures resulted in very slight erythema at nearly all observation time points and desquamation on study day 7, along with very slight edema. Semi-occluded JP-8 exposures showed very slight to slight erythema at most observations, again with desquamation at study day 7. Very slight to slight edema was noted on study days 4 and 7.

HA JP-8 occluded exposures showed very slight erythema at 72 hours and on study days 4, 7 and 14, with desquamation occurring on study day 7; very slight edema was present at study day 7. With the HA JP-8 semi-occluded exposures, very slight erythema was noted during the first 24 hours and very slight to slight edema was present on study days 4, 7 and 14. Again, desquamation was noted on study day 7, as well as very slight to slight edema.

Dermal exposures to KiOR HDCJ were very similar. This alternative fuel produced very slight to slight erythema under both conditions across time points through study day 14, with desquamation on study day 7, under both occluded and semi-occluded conditions. Edema was very slight under semi-occluded conditions and ranged from very slight to slight under occluded conditions.

Occluded ReadiJet exposures also produced very slight erythema at observation points during the first 24 hours and 72 hours through study day 14, with desquamation at study days 7 and 14. Slight edema was noted on day 7. ReadiJet under semi-occluded conditions was found to be

non-irritating, showing only very slight erythema on study days 7 and 14, with desquamation and slight edema on day 7.

The alternative fuel Amyris DSHC occluded exposures resulted in very slight to slight erythema across all observation time points, but showed no instances of desquamation and only a single very slight edema on day 7. Semi-occluded exposures showed only very slight erythema from 48 hours through study day 14, with a single instance of desquamation at day 7 and no edema noted.

Finally, occluded exposures of Virent SAK resulted in erythema ranging from very slight (all rabbits) to severe in one rabbit, with scabbing present at 72 hours and on days 4 and 7, plus desquamation on study day 7 in that individual. Very slight to slight edema was noted on study days 4 and 7. In the semi-occluded exposures, erythema ranged from very slight to slight with desquamation on days 7 and 14 (all rabbits); erythema scores up to moderate were seen in one individual, with scabbing at 72 hours and 4 days, plus desquamation on study day 7.

4.3 Compliance, Quality Assurance and Data Retention

The required compliance and quality assurance statements for Good Laboratory Practices (GLP) can be found in Appendix G. The U.S. Air Force, through the Henry M. Jackson Foundation for the Advancement of Military Medicine, has title to all documentation records, raw data, specimens, or other work product generated during the performance of the study. All remaining work product generated by WIL Research, including raw paper data and specimens, are retained in the WIL Research Archives as specified in the study protocol. Reserve samples of the test substances, pertinent electronic storage media, and the original final report are retained in the WIL Research Archives in compliance with regulatory requirements.

5.0 DISCUSSION AND CONCLUSIONS

Based on PDII scores and descriptive ratings, there were no significant differences in dermal irritation noted between the baseline fuels (JP-8 and HA JP-8) and the four alternate jet fuels when administered dermally to New Zealand white rabbits. All were slightly irritating when exposed occluded and semi-occluded, with the exception of ReadiJet, which was nonirritating when applied under semi-occluded conditions.

Petroleum-derived JP-8 (POSF 4658) has been tested previously, ranging from slightly to moderately irritating (Hurley *et al.*, 2011; Mattie *et al.*, 2013; Sterner *et al.*, 2014). In the current study, JP-8 and HA JP-8 were both found to be slightly irritating; HA JP-8 appears to be somewhat less irritating, per the PDII score. This minor difference is expected as some aliphatic components of JP-8 are more irritating to the skin than aromatic components (Muhammad *et al.*, 2005). In comparing the aliphatic constituents of JP-8 and HA JP-8 (Table 2), normal paraffins are very similar in percentage (by mass), while cycloparaffins are the most different (5.13 percent more in JP-8 than HA JP-8).

While not significantly different from each other, the dermal responses to the alternative fuels measured in this study did indicate that two fuels, Amyris DSHC and Virent SAK, invoke a somewhat stronger irritation reaction than the other fuels (Table 5). Amyris DSHC is composed of 98.90 percent aliphatic compounds, 97.90 percent of that being iso-paraffins (mass percent, Table 2), so aliphatic compounds again appear to have more irritation potential in general. However, this observation does not hold with Virent SAK, which is 97.38 percent aromatics by mass (Table 2). Clearly, the sources of irritation in fuels cannot be associated with just one category of hydrocarbons.

Several alternative fuels have been tested for dermal irritation potential previously. These fuels have had primarily aliphatic components. FT-SPK ranged from nonirritating to moderately irritating (Hurley *et al.*, 2011; Sterner *et al.*, 2014). The complete component analysis for FT-SPK, not previously published, can be found in Appendix H. FT-SPK is 99.84 percent aliphatic constituents by mass. HEFA-SPK formulations from different feedstocks (camelina plant, tallow, animal fats and oils, algae) were tested in this assay by Mattie *et al.* (2013) and Sterner *et al.* (2014). HEFA-SPKs were found to be nonirritating to slightly irritating. HEFA-SPK fuels are also more than 99 percent aliphatic compounds by mass (Mattie *et al.*, 2013). Five additional alternative fuels tested in Sterner *et al.* (2014) were found to range from nonirritating to slightly irritating. In general, alternative fuels made by several different processes from varying feedstocks appear to be similar in dermal irritation potential to petroleum derived JP-8.

In conclusion, this study examined the irritative potential of two petroleum derived operational fuels and four alternative fuels in New Zealand white rabbits. All fuels were found to be equivalent in dermal irritation potential. Based on this assay, it is not expected that normal handling of these fuels would result in increased dermal irritation among airmen.

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APPENDIX A. COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY ANALYSIS AND COMPARISON OF FUELS COMPONENTS

Comparison of Two Baseline JP-8 Fuels with Three Alternative Fuels using GC x GC

Fuel	Baseline JP-8	High Aromatic JP-8 (HA JP-8)	KiOR HDCJ	ReadiJet	Virent SAK
POSF	**4658**	**8457**	**9818**	**10136**	**10326**
Component	**Mass %**	**Mass %**	**Mass %**	**Mass %**	**Mass %**
Aromatics					
Alkylbenzenes					
benzene (C06)	<0.01	0.02	<0.01	<0.01	0.02
toluene (C07)	0.16	0.12	0.57	0.03	0.30
C2-benzene (C08)	0.78	0.75	3.44	1.22	0.68
C3-benzene (C09)	2.24	5.78	3.28	1.56	65.29
C4-benzene (C10)	3.02	7.29	2.34	1.41	22.76
C5-benzene (C11)	2.48	3.24	1.19	1.52	3.59
C6-benzene (C12)	1.93	1.53	0.59	1.25	0.17
C7-benzene (C13)	1.19	0.87	0.12	1.33	<0.01
C8-benzene (C14)	0.89	0.49	0.04	1.32	<0.01
C9+-benzene (C15+)	1.00	0.32	0.53	0.71	<0.01
Total Alkylbenzenes	**13.69**	**20.41**	**12.10**	**10.35**	**92.82**
Diaromatics (Naphthalenes, Biphenyl, etc.)					
diaromatic-C10	0.12	0.96	0.07	0.03	0.02
diaromatic-C11	0.42	0.84	0.20	0.08	<0.01
diaromatic-C12	0.60	0.82	0.29	0.19	<0.01
diaromatic-C13	0.40	0.41	0.19	0.16	<0.01
diaromatic-C14+	0.23	0.23	0.61	0.06	<0.01
Total Alkylnaphthalenes	**1.76**	**3.26**	**1.36**	**0.52**	**0.03**
Cycloaromatics (Indans, Tetralins, etc.)					
cycloaromatic-C09	0.04	0.17	2.46	0.26	0.60
cycloaromatic-C10	0.43	0.95	8.21	1.21	3.02
cycloaromatic-C11	1.13	0.84	10.07	1.64	0.89
cycloaromatic-C12	1.63	1.02	7.06	1.68	0.01
cycloaromatic-C13	1.45	0.90	4.51	1.56	<0.01
cycloaromatic-C14	0.71	0.37	3.31	1.02	<0.01
cycloaromatics-C15+	0.41	0.13	3.92	0.31	<0.01
Total Cycloaromatics	**5.79**	**4.38**	**39.55**	**7.69**	**4.53**
Total Aromatics	**21.25**	**28.05**	**53.02**	**18.56**	**97.38**
Paraffins					
iso-Paraffins					
C07 and lower-iso	0.23	0.17	0.21	<0.01	0.06
C08-isoparaffins	0.56	0.37	0.07	0.13	0.02
C09-isoparaffins	1.08	1.61	0.08	1.05	0.03
C10-isoparaffins	3.59	4.82	0.08	1.36	0.30
C11-isoparaffins	5.12	6.07	0.05	1.37	0.16

C12-isoparaffins	5.31	5.22	0.05	1.19	0.10
C13-isoparaffins	5.25	4.21	0.05	1.17	0.02
C14-isoparaffins	4.44	3.54	<0.01	1.11	<0.01
C15-isoparaffins	3.10	2.09	<0.01	0.80	<0.01
C16-isoparaffins	1.66	0.72	<0.01	0.36	<0.01
C17-isoparaffins	0.69	0.26	<0.01	0.07	<0.01
C18-isoparaffins	0.19	0.08	<0.01	0.01	<0.01
C19-isoparaffins	0.08	0.03	<0.01	<0.01	<0.01
C20-isoparaffins	0.02	<0.01	<0.01	<0.01	<0.01
C21-isoparaffins	<0.01	<0.01	<0.01	<0.01	<0.01
C22-isoparaffins	<0.01	<0.01	<0.01	<0.01	<0.01
C23-isoparaffins	<0.01	<0.01	<0.01	<0.01	<0.01
C24-isoparaffins	<0.01	<0.01	<0.01	<0.01	<0.01
Total iso-Paraffins	**31.34**	**29.21**	**0.61**	**8.63**	**0.72**
n-Paraffins					
n-C07 and lower	0.15	0.17	<0.01	0.09	0.13
n-C08	0.54	0.50	<0.01	2.78	<0.01
n-C09	1.14	1.66	0.05	6.45	0.07
n-C10	2.55	3.80	<0.01	5.24	0.11
n-C11	3.62	4.06	0.02	5.59	0.05
n-C12	3.70	3.58	0.02	3.24	0.02
n-C13	2.86	2.72	<0.01	2.21	<0.01
n-C14	2.17	1.81	<0.01	1.68	<0.01
n-C15	1.28	0.78	<0.01	1.87	<0.01
n-C16	0.61	0.26	<0.01	0.88	<0.01
n-C17	0.27	0.10	<0.01	0.07	<0.01
n-C18	0.05	0.02	<0.01	0.03	<0.01
n-C19	0.02	<0.01	<0.01	<0.01	<0.01
n-C20	<0.01	<0.01	<0.01	<0.01	<0.01
n-C21	<0.01	<0.01	<0.01	<0.01	<0.01
n-C22	<0.01	<0.01	<0.01	<0.01	<0.01
n-C23	<0.01	<0.01	<0.01	<0.01	<0.01
Total n-Paraffins	**19.00**	**19.46**	**0.12**	**30.18**	**0.39**
Cycloparaffins					
Monocycloparaffins					
C07 and lower-monocycloparaffins	0.20	0.27	1.14	0.07	0.04
C08-monocycloparaffins	0.69	0.48	4.05	1.79	0.03
C09-monocycloparaffins	1.67	1.60	5.44	4.83	0.14
C10-monocycloparaffins	3.26	3.26	4.30	5.04	0.51
C11-monocycloparaffins	4.11	3.83	2.80	5.40	0.24
C12-monocycloparaffins	4.07	3.04	1.24	4.06	0.03
C13-monocycloparaffins	3.65	3.16	0.57	4.24	<0.01
C14-monocycloparaffins	2.43	1.77	0.43	4.25	<0.01
C15-monocycloparaffins	1.55	0.86	0.19	2.42	<0.01
C16-monocycloparaffins	0.64	0.23	0.07	0.68	<0.01
C17-monocycloparaffins	0.28	0.07	0.04	0.11	<0.01
C18-monocycloparaffins	0.06	0.01	0.01	0.05	<0.01
C19+-monocycloparaffins	0.03	<0.01	<0.01	<0.01	<0.01
Total Monocycloparaffins	**22.64**	**18.58**	**20.29**	**32.95**	**0.99**
Dicycloparaffins (Decalins, Bihexyls, etc.)					

C08-dicycloparaffins	0.02	0.02	0.02	0.10	<0.01
C09-dicycloparaffins	0.29	0.16	3.48	1.39	0.24
C10-dicycloparaffins	0.43	0.58	4.75	1.75	0.17
C11-dicycloparaffins	1.26	1.11	4.33	1.71	0.10
C12-dicycloparaffins	1.22	1.07	3.39	1.87	0.01
C13-dicycloparaffins	1.42	1.03	2.10	1.28	<0.01
C14-dicycloparaffins	0.82	0.47	1.90	1.02	<0.01
C15-dicycloparaffins	0.21	0.11	1.05	0.42	<0.01
C16-dicycloparaffins	0.02	0.02	0.62	0.09	<0.01
C17+-dicycloparaffins	0.03	0.01	0.65	0.02	<0.01
Total Dicycloparaffins	**5.73**	**4.58**	**22.28**	**9.66**	**0.52**
Tricycloparaffins					
C10-tricycloparaffins	<0.01	<0.01	<0.01	<0.01	<0.01
C11-tricycloparaffins	0.05	0.10	<0.01	0.02	<0.01
C12-tricycloparaffins	<0.01	0.03	0.15	<0.01	<0.01
C13-tricycloparaffins			0.83		<0.01
C14-tricycloparaffins			1.00		<0.01
C15-tricycloparaffins			0.73		<0.01
C16-tricycloparaffins			0.30		<0.01
C17-tricycloparaffins			0.66		<0.01
Total Tricycloparaffins	**0.05**	**0.12**	**3.68**	**0.02**	**<0.01**
Total Cycloparaffins	**28.42**	**23.29**	**46.25**	**42.63**	**1.51**
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Average Molecular Formula - C	11.69	11.11	10.71	11.03	9.32
Average Molecular Formula - H	22.62	20.89	16.89	21.32	12.71

GC x GC Analysis of Amyris C15 DSHC Alternative Fuel

Fuel	**Amyris C15 DSHC (farnesane)**
POSF	**10198, 10324, 10323**
Component	**Mass %**
Trimethyl dodecane (C15 isoparaffin)	97.10
Trimethyl dodecanol	1.00
C15 Cyclo-aromatics and Aromatics	1.10
C28-C30	0.40
Other C15 compounds	0.40
TOTAL	**100.00**

APPENDIX B. STUDY PROTOCOL, AMENDMENT AND DEVIATION

DEVIATIONS FROM THE PROTOCOL

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 5.8** states that each animal will be uniquely identified by a plastic ear tag displaying the animal number. However, the animals were identified using BMDS microchips.

  Impact Assessment: This deviation had no impact because the animals were still uniquely identified.

This deviation did not negatively impact the quality or integrity of the data or the outcome of the study.

Study Number: WIL-773004

PROTOCOL AMENDMENT 1

Sponsor: The Henry M. Jackson Foundation for the Advancement of Military Medicine

Title of Study:

Acute Dermal Irritation Study of Four Alternative Jet Fuels Plus Two Baseline JP-8 Fuels in New Zealand White Rabbits

Protocol Modifications:

1) **3 STUDY SCHEDULE**
The section is replaced with the following:
Proposed Experimental Start Date: 08 October 2013
Proposed Experimental Termination Date: 22 October 2013
Proposed Audited Draft Report Date: 03 December 2013

Reason for Protocol Modification:
1) Study scheduled was to be added by amendment.

Approval:

Sponsor's approval was obtained via e-mail on 23 September 2013.

The Henry M. Jackson Foundation for the Advancement of Military Medicine

David R. Mattie 30 Sept 13

David R. Mattie, PhD, DABT
Sponsor Representative

Date

WIL Research

01 Oct 2013

Jonathan M. Hurley, BS
Study Director

Date

WIL-773004
23 August 2013

PROTOCOL

Acute Dermal Irritation Study of Four Alternative Jet Fuels Plus Two Baseline JP-8 Fuels in New Zealand White Rabbits

Submitted To:

The Henry M. Jackson Foundation
for the Advancement of Military Medicine
1401 Rockville Pike, Suite 600
Rockville, MD 20852

WIL Research
1407 George Road
Ashland, OH 44805-8946

1 OBJECTIVE:

To determine the irritative potential of the test substances following a single exposure to the skin of albino rabbits.

This protocol has been designed and the study will be conducted in general compliance with the following guidelines:

> Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substance (OPPTS) guideline 870.2500 (1998).
>
> Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 404 (2002).
>
> The European Union (EU) Guideline in the Official Journal of the European Communities [92/69, Annex V, B4 (1992)].

The study will be conducted in compliance with the U.S. EPA Good Laboratory Practices (40 CFR Part 792); with the exception that analytical confirmation of the concentration, homogeneity and stability of the dosing mixture (if prepared) will not be performed.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Sponsor Representative:

> David R. Mattie, PhD, DABT
> Molecular Bioeffects Branch
> 711 HPW/RHDJ
> 2729 R Street, Bldg 837
> Wright-Patterson AFB, OH 45433-5707
> Phone: (937) 904-9569
> Email: David.Mattie@wpafb.af.mil

2.2 WIL Study Director:

> Jonathan M. Hurley, BS
> Staff Toxicologist and Head of Acute Toxicology
> Phone: (419) 289-8700
> Fax: (419) 289-3650
> E-mail: jon.hurley@wilresearch.com

2.3 WIL Departmental Responsibilities:

Teresa D. Morris, BS
Assistant Director, General Toxicology
Emergency Contact
Phone: (419) 289-8700
E-mail: teresa.morris@wilresearch.com

Donald G. Stump, PhD, DABT
Vice President, Non-Clinical Safety Sciences, U.S.

Howard E. Moody, MS
Vice President, Chief Information Officer

Alex K. Eapen, PhD, DABT
Associate Director, General Toxicology

Sally A. Keets, AS
Senior Operations Manager, Central Scheduling

Erica L. Lashley, BS, LAT
Senior Animal Operations Manager

Bryan P. Fennel, BS
Group Manager, Formulations Laboratory

Patrick A. Swyers, BS
Manager, Gross Pathology Toxicology Laboratory

Gwendalyn M. Maginnis, DVM
Attending Veterinarian

Robert A. Wally, BS
Operations Manager, Reporting
& Technical Support Services

Heather L. Johnson, BS, RQAP-GLP
Assistant Director of Quality and Regulatory Compliance

3 STUDY SCHEDULE:

Proposed Experimental Start Date:	To be added by amendment
Proposed Experimental Termination Date:	To be added by amendment
Proposed Audited Draft Report Date:	To be added by amendment

4 TEST ARTICLES:

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Information on composition and method of synthesis will be held by the Sponsor. Test Substances #1 and #2 are operational fuels with which the alternative fuels will be compared. Test Substances #3-6 are alternative fuels for dermal testing.

4.1 Test Article #1 Identification I Lot Number:

25% petroleum aromatic JP-8 fuel as a testing baseline / Lot no. 8457
Label Identification: 25% Aromatic JP-8

4.2 Test Article #2 Identification I Lot Number:

JP-8 as dermal irritation baseline / Lot no. POSF 4658
Label Identification: JP-8 POSF 4658

4.3 Test Article #3 Identification I Lot Number:

KiOR Hydroprocessed Depolymerized Cellulosic Jet (HDCJ) / Lot no. 10327
Label Identification: KiOR 9818 w/JP-8 Additive

4.4 Test Article #4 Identification / Lot Number:

Applied Research Associates "ReadiJet." / Lot no.10328
Label Identification: 10136 w/JP-8 Additives

4.5 Test Article #5 Identification / Lot Number:

Amyris C15 (farnesane) Direct Sugar To Hydrocarbon (DSHC) / Lot no. 10329.
Label Identification: 10150 w/JP-8 Additives

4.6 Test Article #6 Identification I Lot Number:

Virent Synthetic Aromatic Kerosene (SAK) / Lot no.10330
Label Identification: 10326 w/JP-8 Additives

4.7 Purity:

Responsibility of the Sponsor

4.8 Stability:

Considered to be stable for years when properly stored.

4.9 Physical Description:

To be documented by WIL Research

4.10 Storage Conditions:

Store at room temperature. Keep containers closed tightly. Use and store these materials in cool, dry, well-ventilated areas away from heat, direct sunlight, hot metal surfaces and all sources of ignition.

4.11 Personnel Safety:

At minimum, appropriate gloves, eye protection and long sleeves (lab coat) are to be worn during dose administration. Refer to Material Safety Data Sheets for complete available information.

4.12 Retention Safety:

Retention samples of the test substances (as received) will be collected in accordance with WIL Research SOP No. T2-001.

4.13 Unused Test Substances:

Unused portions of the test substances will be returned following the issuance of the final study report to the contact below.

David R. Mattie, PhD, DABT
711 HPW/RHDJ
2729 R Street, Bldg 837
Wright-Patterson AFB, OH 45433-5707
Phone: (937) 904-9569
Email: David.Mattie@WPAFB.AF.MIL

5 TEST SYSTEM:

5.1 Species:

Albino rabbit *(Oryctolagus cuniculus)*

5.2 Breed:

New Zealand White

5.3 Source:

Covance Research Products, Inc. (USDA License # 23-A-0180)
(Documentation of the specific breeding facility will be maintained in the study records and included in the final report.)

5.4 Number on Stud:

Six animals from the acute stock colony

5.6 Body Weight Range:

2.0 kg or greater

5.7 Approximate Age:

Young adult, at least 12 weeks old at initiation of dosing

5.8 Identification System:

Each animal will be uniquely identified by a plastic ear tag displaying the animal number. Individual cage cards will be affixed to each cage and will display the animal number, group and study number.

5.9 Justification for Selection:

This species and breed is generally recognized as appropriate for acute dermal irritation studies. The number of animals selected is the minimum required to satisfy regulatory guidelines. The experimental design uses the procedures and standards required by the current federal and international regulations.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

The animals will be housed individually in stainless steel cages in an environmentally controlled room. Animals will be housed in clean cages elevated above ground corncob bedding or other suitable material that will be changed at least twice each week. Animals will be changed out into clean cages approximately every two weeks. The facilities at WIL Research are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

6.2 Environmental Conditions:

Controls will be set to maintain the temperature at 66 ± 5°F (19 ± 3°C) and the relative humidity at 50 ± 20%. Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic collection on an hourly basis. Fluorescent lighting controlled by light timers will provide illumination for a 12-hour light/dark photoperiod. Temporary adjustments to the light/dark cycles may be made to accommodate protocol specified activities. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

6.3 Drinking Water:

Municipal water will be available *ad libitum*. Filters servicing the automatic watering system will be changed regularly according to Standard Operating Procedures (SOPs). Municipal water supplying the laboratory is analyzed for contaminants according to SOPs to ascertain that none are present at concentrations that would be expected to affect the outcome of the study and the results are maintained on file.

6.4 Basal Diet:

PMI Nutrition International, LLC Certified High Fiber Rabbit LabDiet® 5325 will be offered at approximately 150 g/day during the study. The amount of feed provided will be estimated and will not be documented. Standard Operating Procedures provide specifications for acceptable levels of heavy metals and pesticides that are reasonably expected to be present in the diet without interfering with the purpose or conduct of the study. Analyses are performed and provided by the manufacturer and the results are maintained on file.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Acclimation:

Animals selected for this study were/will be received/transferred into and maintained in the acute stock colony according to WIL Standard Operating Procedures. Animals will be acclimated to laboratory conditions for a minimum of 5 days, following receipt, prior to selection for use on the study.

All records and data collected while the animals are in the stock colony will be maintained in the stock colony records. Copies of records of receipt, identification and any specific procedures performed that are related to the actual conduct of the study will be included in the study records.

7.2 Veterinary Care:

Animals will be monitored by the technical staff for any condition requiring possible veterinary care. If any such condition is identified, the veterinary staff will be notified for an examination and evaluation. Animals will be treated as outlined in the Animal Welfare Act Compliance section of the protocol.

7.3 Route and Rationale of Test Article Administration:

The route of administration will be dermal (clipped, intact skin) in order to evaluate the dermal irritation potential of the test substances. This study is intended to provide information on the health hazards likely to arise from a short-term exposure to the test substances by the dermal route.

7.4 Organization of Treatment Groups:

Following the acclimation period, animals will be arbitrarily selected from available stock based upon health and body weight and assigned to 2 groups of 3 rabbits/group as shown below. No separate control group will be utilized; each animal will serve as its own control. The skin of all test sites will be left intact (unabraded).

Group Number	Test Substances*	Dose Volume (mL/Test Substance)	Exposure Method	Number of Males
1	#1, #2, #3, #4, #5, #6	0.5	Occluded	3
2	#1, #2, #3, #4, #5, #6	0.5	Semi-occluded	3

*#1 = 25% petroleum aromatic JP-8 fuel as a testing baseline; #2 = JP-8 as dermal irritation baseline, #3 = KiOR Hydroprocessed Depolymerized Cellulosic Jet (HDCJ), #4 = Applied Research Associates "ReadiJet.", #5 = Amyris C15 (farnesane) Direct Sugar To Hydrocarbon (DSHC), #6 = Virent Synthetic Aromatic Kerosene (SAK)

7.5 Test Material Preparation:

The test articles will be administered undiluted as received at a dosage of 0.5 mL. The pH will be determined and recorded.

7.6 Animal Preparation

On the day prior to dermal applications, the back and flanks of each animal will be clipped free of hair with a small animal clipper. The clipped area on each animal will constitute approximately 20-25% of the total body surface area (actual size of area will not be recorded). Animals with dermal abnormalities or injuries will be excluded.

7.7 Method of Administration

Six sites located lateral to the midline of the back will be selected on each rabbit. The location of the test sites (designated A-F based upon six available site locations on the back of the rabbit) will be rotated so that no test substance is applied to the same site within a group of rabbits. The test sites will be delineated with four dots made with indelible ink spaced approximately 2.5 centimeters apart arranged in a square. All animals will receive a single application of six test substances.

Each test site will be immediately covered with a two ply, 2.5-cm square gauze patch. The patch will be secured in place with surgical porous tape. For animals in the occluded exposure groups the trunk of the animal will be wrapped with plastic wrap to occlude the test site. The trunk of animals in both the occluded and semi-occluded groups will then be wrapped with gauze bandaging that will be secured with several wrappings of non-irritating tape. Elizabethan collars will be applied to each animal during the exposure period to prevent ingestion of the test substances and/or wrappings.

After the four hours of exposure, the bandages will be removed and residual test substance cleansed from the application sites using clean, disposable paper towels moistened with deionized water (as thoroughly as possible without irritating the skin). The same towel will not be used on more than one site.

8 OBSERVATIONS:

8.1 Viability and Clinical Observations:

All animals will be observed for mortality/moribundity twice daily (morning and afternoon) for the duration of the study. Moribund animals will be removed from study and euthanized by intravenous injection of sodium pentobarbital. All animals will receive a detailed physical examination on the day of dosing.

8.2 Dermal Observations:

Approximately 30-60 minutes after test substance removal, each test site will be examined and the degree of erythema and edema recorded according to the Draize technique (Appendix). The presence of any other dermal findings will also be recorded. Additional examinations will be performed at approximately 24, 48 and 72 hours after patch removal. If no irritation is present at the 72-hour observation, the study may be terminated.

If irritation is present at the end of 72 hours, additional observations will be performed on days 4, 7 and 14, or until irritation subsides. The study need not normally exceed 14 days after application unless specifically requested and authorized by the Sponsor. Individual animals will be terminated if no irritation is present at the 72-hour or any subsequent observation. At the request of the Sponsor, observations may be terminated prior to 14 days and/or resolution of irritation.

The areas of application will be clipped free of hair a minimum of one hour before scoring, as needed during the study, to facilitate accurate dermal observations.

8.3 Body Weights:

The body weight of each animal will be determined on study day 0 and at termination.

8.4 Gross Pathology:

All animals will be euthanized by intravenous injection of sodium pentobarbital. A gross necropsy examination on major organ systems of the thoracic and visceral cavities will be conducted on all animals found dead or euthanized *in extremis*. Animals euthanized following study termination will be discarded without further examination.

9 CALCULATION OF THE PRIMARY DERMAL IRRITATION INDEX:

The Primary Dermal Irritation Index will be calculated from the scores recorded at 30-60 minutes, 24, 48 and 72 hours (after patch removal). The mean scores for erythema and edema will be calculated separately to the nearest tenth and added together. Based on this value, the grading system in the Appendix will be used to arrive at a primary dermal irritation descriptive rating for each test article for the occluded and unoccluded method of exposure.

10 REPORT:

The final report will include, but will not necessarily be limited to, the following: compliance statement, summary, objective, test article identification and receipt information, methods, observations, mortality, body weights, individual and summarized

dermal scores/findings, classification of the test articles based on their dermal irritation properties, results and discussion, key personnel, a signed QAU statement and protocol deviation(s), if any.

WIL Research will submit one electronic copy (PDF with an MS Word copy of the report text for editing and comments) of an audited draft report in a timely manner upon completion of data collection prior to issuance of the final report. It is expected that the Sponsor will review the draft report and provide comments to WIL Research within a two-month time frame following submission. Within one month following receipt of the Sponsor's comments, WIL Research shall provide a revised draft report that incorporates the Sponsor's reasonable revisions and suggestions. One revision will be permitted as part of the cost of the study; additional changes or revisions may be made, at extra cost. WIL Research will submit the final report within two weeks of receiving authorization from the Sponsor. If the Sponsor's comments and/or authorization to finalize the report have not been received at WIL Research within one year following submission of the draft report, WIL Research may elect to finalize the report following appropriate written notification to the Sponsor. An electronic copy (hyperlinked and bookmarked PDF) of the final report will be provided. Requests for paper copies of the final report may result in additional charges.

11 RECORDS TO BE MAINTAINED:

All original raw data records (as defined by the applicable GLPs and WIL Research SOPs) generated by WIL Research will be collected and maintained in the WIL Research Archives as described in the following section.

12 WORK PRODUCT:

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including raw paper data, pertinent electronic storage media and specimens will be retained at no charge for a period of six months following issuance of the final report in the WIL Research Archives. Thereafter, WIL Research will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research to another location will be appropriately packaged and labeled as defined by WIL Research's SOPs and delivered to a common carrier for shipment. WIL Research will not be responsible for shipment following delivery to the common carrier.

13 QUALITY ASSURANCE:

The study will be audited by the WIL Research Quality Assurance Department while in progress to assure compliance with applicable Good Laboratory Practices and adherence to the protocol and to WIL Research SOPs. The raw data and draft report will be audited by the WIL Research Quality Assurance Department to assure that the final report accurately describes the conduct and the findings of the study.

14 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

15 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

- The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that, for the study described in this protocol, there are no acceptable non-animal alternatives and the study does not unnecessarily duplicate previous experiments.
- Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory SOPs.
- Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.
- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.
- The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

16 PROTOCOL APPROVAL:

Sponsor approval received by the Study Director via e-mail on 21 August 2013.

The Henry M. Jackson Foundation for the Advancement of Military Medicine

David R. Mattie — 23 Aug 13

David R. Mattie, PhD, DABT
Sponsor Representative

Date

WIL Research

Jonathan M. Hurley — 23 Aug 2013

Jonathan M. Hurley, BS
Study Director

Date

APPENDIX

SCORING CRITERIA FOR DERMAL REACTIONS*

Value	Erythema and Eschar Formation
0	No erythema
1	Very slight erythema (barely perceptible, edges of area not well defined)
2	Slight erythema (pale red in color and edges definable)
3	Moderate to severe erythema (definite red in color and area well defined)
4	Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth)
4	Maximum possible erythema score

	Edema Formation
0	No edema
1	Very slight edema (barely perceptible, edges of area not well defined)
2	Slight edema (edges of area well defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)
4	Maximum possible edema score
8	Maximum total possible Primary Irritation Score

DESCRIPTIVE RATINGS

Mean Primary Dermal Irritation Index

Range of Values	Descriptive Rating
0	Nonirritating
0.1 - 2.0	Slightly Irritating
2.1 - 5.0	Moderately Irritating
5.1 - 8.0	Severely Irritating

*Draize, J.H., 1965. The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Dermal Toxicity, pp. 46-59. Assoc. of Food and Drug Officials of the U.S., Topeka, Kansas and the EPA-OPPTS Health Effects Test Guidelines (1998).

APPENDIX C. SCORING CRITERIA FOR DERMAL REACTIONS

Evaluation of Dermal Reactions[a]

Value	Erythema and Eschar Formation
0	No erythema
1	Very slight erythema (barely perceptible, edges of area not well defined)
2	Slight erythema (pale red in color and edges definable)
3	Moderate to severe erythema (definite red in color and area well defined)
4	Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth)
4	Maximum possible erythema score

Value	Edema Formation
0	No edema
1	Very slight edema (barely perceptible, edges of area not well defined)
2	Slight edema (edges of area well defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)
4	Maximum possible edema score

8 Maximum total possible Primary Irritation Score

Descriptive Ratings

Mean Primary Dermal Irritation Index Range of Values	Descriptive Rating
0	Nonirritating
0.1 - 2.0	Slightly Irritating
2.1 - 5.0	Moderately Irritating
5.1 - 8.0	Severely Irritating

[a]Draize, J.H., 1965. The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Dermal Toxicity, pp. 46-59. Assoc. of Food and Drug Officials of the U.S., Topeka, Kansas and the EPA-OPPTS Health Effects Test Guidelines (1998).

APPENDIX D. DATA ACQUISITION AND REPORTING SYSTEMS

Program/System	Description
Archive Management System (AMS)	In-house developed application for storage, maintenance, and retrieval information for archived materials (*e.g.*, lab books, study data, wet tissues, slides, etc.)
Bio Medic Data Systems (BMDS) Implantable Programmable Temperature Transponders™ (IPTT 300)	Animal identification
Formulations Dose Dispensing Management System (FDDMS)	In-house developed system used to assign unique barcodes to formulation containers and individual containers used for dispensing dosing formulations.
InSight® Publisher	Electronic publishing system (output is Adobe Acrobat, PDF)
Master Schedule	Maintains the master schedule for the company.
Metasys DDC Electronic Environmental Control System	Controls and monitors animal room environmental conditions.
Microsoft® Office 2007	Used in conjunction with the publishing software to generate study reports.
Provantis Dispense™	Comprehensive system (Instem LSS Limited) to manage test materials, including receipt, formulation instructions, and accountability.
WIL Metasys	In-house developed system used to record and report animal room environmental conditions.

Note: Version numbers of WTDMS™ programs used for the study are presented on the report data tables (reporting programs); version numbers and release dates are otherwise maintained in the study records and/or facility records.

APPENDIX E. ANIMAL ROOM ENVIRONMENTAL CONDITIONS

ACUTE DERMAL IRRITATION STUDY OF JET AND JP-8 FUELS IN RABBITS

PROJECT NO.:WIL- 773004 TEMPERATURE/HUMIDITY - STUDY SUMMARY REPORT

SPONSOR: 773 - E M JACKSON FOUNDATION

STUDY SPECIFICATIONS: 773004 DATE IN 10/08/13 TIME IN 08:00

DATE OUT 10/23/13 TIME OUT 16:00

ROOM SPECIFICATIONS: B ROOM 37 LOW TEMPERATURE °F: 61.0 HIGH TEMPERATURE °F: 71.0 LOW HUMIDITY %RH: 30.0

TEST SYSTEM: RABBIT LOW TEMPERATURE °C: 16.1 HIGH TEMPERATURE °C: 21.7 HIGH HUMIDITY %RH: 70.0

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
DATE	MEAN (°F)	MEAN (°C)	MEAN (°F)	MEAN (°C)	MEAN (%RH)	MEAN (%RH)
10/08/13	65.4	18.6	65.2	18.4	48.9	49.9
10/09/13	65.5	18.6	65.3	18.5	47.5	48.6
10/10/13	65.6	18.7	65.4	18.6	47.8	48.8
10/11/13	65.5	18.6	65.3	18.5	48.3	49.5
10/12/13	65.5	18.6	65.3	18.5	50.2	51.2
10/13/13	65.4	18.6	65.3	18.5	52.1	52.7
10/14/13	65.5	18.6	65.3	18.5	49.5	50.4
10/15/13	65.7	18.7	65.5	18.6	51.2	51.9
10/16/13	65.5	18.6	65.4	18.6	52.6	53.3
10/17/13	65.6	18.7	65.4	18.6	48.7	49.7
10/18/13	65.5	18.6	65.3	18.5	46.1	47.4
10/19/13	65.6	18.7	65.3	18.5	45.3	46.7
10/20/13	65.4	18.6	65.2	18.4	45.2	46.7
10/21/13	65.7	18.7	65.5	18.6	46.0	47.5
10/22/13	65.9	18.8	65.7	18.7	43.7	45.2
10/23/13	65.3	18.5	65.0	18.3	44.6	46.3

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
DATE	MEAN (°F)	MEAN (°C)	MEAN (°F)	MEAN (°C)	MEAN (%RH)	MEAN (%RH)

SUMMARY OF DAILY MEANS	MEAN	MIN	MAX
PRIMARY TEMP °F:	65.5	65.3	65.9
PRIMARY TEMP °C:	18.6	18.5	18.8
SECONDARY TEMP °F:	65.3	65.0	65.7
SECONDARY TEMP °C:	18.5	18.3	18.7
PRIMARY HUM %RH:	48.0	43.7	52.6
SECONDARY HUM %RH:	49.1	45.2	53.3
N DAYS	16		

B ROOM 37 SUMMARY OF HOURLY VALUES

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
MEAN	65.5 °F	18.6 °C	65.3 °F	18.5 °C	48.0 %RH	49.1 %RH
MIN	63.6 °F	17.6 °C	63.3 °F	17.4 °C	39.0 %RH	41.4 %RH
MAX	67.4 °F	19.7 °C	66.9 °F	19.4 °C	66.3 %RH	64.5 %RH
SD	0.58	0.32	0.61	0.34	3.49	3.12
SE	0.03	0.02	0.03	0.02	0.18	0.16
N SAMPLES	368		368		368	368
FIRST DAY	10/08/13					
LAST DAY	10/23/13					
N DAYS	16					

STUDY 773004 SUMMARY OF HOURLY VALUES

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
MEAN	65.5 °F	18.6 °C	65.3 °F	18.5 °C	48.0 %RH	49.1 %RH
MIN	63.6 °F	17.6 °C	63.3 °F	17.4 °C	39.0 %RH	41.4 %RH
MAX	67.4 °F	19.7 °C	66.9 °F	19.4 °C	66.3 %RH	64.5 %RH
SD	0.58	0.32	0.61	0.34	3.49	3.12
SE	0.03	0.02	0.03	0.02	0.18	0.16
N SAMPLES	368		368		368	368
FIRST DAY	10/08/13					
LAST DAY	10/23/13					
N DAYS	16					

APPENDIX F. INDIVIDUAL DERMAL DATA

TABLE 1
INDIVIDUAL DERMAL SCORES

Material: 25% petroleum aromatic JP-8 fuel as a testing baseline, Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2964	M	A	0	0	0	1	1	1	0	0	0	0	0	0	0	0
2965	M	B	0	0	0	0	0	1d	1	0	0	0	0	0	1	0
2966	M	C	0	0	0	1	1	1d	1	0	0	0	0	0	1	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 0 + 0 + 2)/ 12 + (0 + 0 + 0 + 0) / 12

PII = 2 / 12 + 0 / 12

PII = 0.2 + 0.0

PII = 0.2 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation

Site Locations: Head

A D

B E

C F

Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 2**

Material: 25% petroleum aromatic JP-8 fuel as a testing baseline, Semi-Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2967	M	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2968	M	E	0	0	0	0	1	1d	1	0	0	0	0	0	1	0
2969	M	F	1	1	0	0	1	2d	2	0	0	0	0	0	2	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (1 + 1 + 0 + 0)/ 12 + (0 + 0 + 0 + 0) / 12
PII = 2 / 12 + 0 / 12
PII = 0.2 + 0.0
PII = 0.2 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation

Site Locations:

Head
A D
B E
C F
Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 3**

Material: JP-8 as dermal irritation baseline, Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2964	M	B	1	1	1	1	1	1d	1	0	0	0	0	0	1	0
2965	M	C	1	1	0	1	1	1d	1	0	0	0	0	0	1	0
2966	M	D	0	0	0	0	0	0d	0	0	0	0	0	0	0	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (2 + 2 + 1 + 2)/ 12 + (0 + 0 + 0 + 0) / 12
PII = 7 / 12 + 0 / 12
PII = 0.6 + 0.0
PII = 0.6 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation

Site Locations:

Head
A D
B E
C F
Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 4**

Material: JP-8 as dermal irritation baseline, Semi-Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2967	M	E	0	0	0	0	1	1d	1	0	0	0	0	0	0	0
2968	M	F	1	1	1	1	2	2d	1	0	0	0	0	1	2	0
2969	M	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (1 + 1 + 1 + 1)/ 12 + (0 + 0 + 0 + 0) / 12

PII = 4 / 12 + 0 / 12

PII = 0.3 + 0.0

PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation

Site Locations:

Head

A D

B E

C F

Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 5**

Material: KiOR Hydroprocessed Depolymerized Cellulosic Jet (HDCJ), Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2964	M	C	1	2	1	1	2	1d	1	0	0	0	0	1	2	0
2965	M	D	0	0	0	0	0	1d	1	0	0	0	0	0	1	0
2966	M	E	0	0	0	0	1	1d	1	0	0	0	0	0	1	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (1 + 2 + 1 + 1)/ 12 + (0 + 0 + 0 + 0) / 12
PII = 5 / 12 + 0 / 12
PII = 0.4 + 0.0
PII = 0.4 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation

Site Locations:

Head
A D
B E
C F
Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 6**

Material: KiOR Hydroprocessed Depolymerized Cellulosic Jet (HDCJ), Semi-Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2967	M	F	1	1	1	1	1	2d	2	0	0	0	0	0	1	0
2968	M	A	0	0	0	0	0	1d	0	0	0	0	0	0	1	0
2969	M	B	0	0	0	0	0	1d	1	0	0	0	0	0	1	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (1 + 1 + 1 + 1)/ 12 + (0 + 0 + 0 + 0) / 12
PII = 4 / 12 + 0 / 12
PII = 0.3 + 0.0
PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation

Site Locations:

Head
A D
B E
C F
Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 7**

Material: Applied Research Associates "ReadiJet", Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2964	M	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2965	M	E	1	1	0	1	1	1	1d	0	0	0	0	0	1	0
2966	M	F	0	0	0	1	1	1d	1	0	0	0	0	0	1	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (1 + 1 + 0 + 2)/ 12 + (0 + 0 + 0 + 0) / 12
PII = 4 / 12 + 0 / 12
PII = 0.3 + 0.0
PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation

Site Locations:

Head
A D
B E
C F
Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 8**

Material: Applied Research Associates "ReadiJet", Semi-Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2967	M	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2968	M	B	0	0	0	0	0	0d	0	0	0	0	0	0	1	0
2969	M	C	0	0	0	0	0	1d	1	0	0	0	0	0	1	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (0 + 0 + 0 + 0)/ 12 + (0 + 0 + 0 + 0) / 12
PII = 0 / 12 + 0 / 12
PII = 0.0 + 0.0
PII = 0.0 = Nonirritating

M = Male; H = Hours; D = Day; d = Desquamation

Site Locations:

Head
A D
B E
C F
Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 9**

Material: Amyris C15 (farnesane) Direct Sugar To Hydrocarbon (DSHC), Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2964	M	E	1	2	1	1	2	2	1	0	0	0	0	0	0	0
2965	M	F	1	1	0	1	1	2	1	0	0	0	0	0	1	0
2966	M	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (2 + 3 + 1 + 2)/ 12 + (0 + 0 + 0 + 0) / 12
PII = 8 / 12 + 0 / 12
PII = 0.7 + 0.0
PII = 0.7 = Slightly Irritating

M = Male; H = Hours; D = Day

Site Locations:

Head
A D
B E
C F
Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 10**

Material: Amyris C15 (farnesane) Direct Sugar To Hydrocarbon (DSHC), Semi-Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2967	M	B	0	0	1	1	1	1d	1	0	0	0	0	0	0	0
2968	M	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2969	M	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (0 + 0 + 1 + 1)/ 12 + (0 + 0 + 0 + 0) / 12
PII = 2 / 12 + 0 / 12
PII = 0.2 + 0.0
PII = 0.2 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation

Site Locations:

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 11**

Material: Virent Synthetic Aromatic Kerosene (SAK), Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2964	M	F	1	2	4	1s	1s	1ds	1	0	0	0	0	1	2	0
2965	M	A	0	0	0	0	0	1d	1	0	0	0	0	0	2	0
2966	M	B	0	0	0	0	0	1d	0	0	0	0	0	0	1	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (1 + 2 + 4 + 1)/ 12 + (0 + 0 + 0 + 0) / 12
PII = 8 / 12 + 0 / 12
PII = 0.7 + 0.0
PII = 0.7 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation; s = Scabbing

Site Locations:

Head
A D
B E
C F
Tail

TABLE 1
INDIVIDUAL DERMAL SCORES **PAGE 12**

Material: Virent Synthetic Aromatic Kerosene (SAK), Semi-Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
2967	M	C	1	1	1	1	1	1d	2	0	0	0	0	0	0	0
2968	M	D	0	0	1	1	1	1d	1d	0	0	0	0	0	1	0
2969	M	E	1	2	3	2s	2s	2d	2	1	1	1	1	1	2	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H
Primary Irritation Index (PII) = (2 + 3 + 5 + 4)/ 12 + (1 + 1 + 1 + 1) / 12
PII = 14 / 12 + 4 / 12
PII = 1.2 + 0.3
PII = 1.5 = Slightly Irritating

M = Male; H = Hours; D = Day; d = Desquamation; s = scabbing

Site Locations:

Head
A D
B E
C F
Tail

APPENDIX G. COMPLIANCE AND QUALITY ASSURANCE STATEMENTS

WIL-773004 Four Alternative Jet Fuels Plus Two Baseline JP-8 Fuels
The Henry M. Jackson Foundation

COMPLIANCE STATEMENT

This study, designated WIL-773004, was conducted in compliance with the United States EPA Good Laboratory Practices (40 CFR 792), 18 September 1989; WIL Research's SOPs; and the protocol as approved by the Sponsor with the following exception. Analytical confirmation of the concentration, purity, homogeneity, and stability of the test substances was not supplied by the Sponsor and was not conducted as part of this study. Due to the screening nature of this study and the inherent stability of jet fuels, this exception did not negatively impact the quality or integrity of the data or interpretation of the results of the study.

Jonathan M. Hurley, BS
Staff Toxicologist &
Head of Acute Toxicology

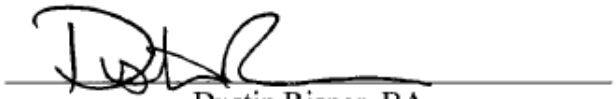
09 Jan 2014
Date

QUALITY ASSURANCE STATEMENT

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director and Management
23-Aug-2013	Protocol	23-Aug-2013
01-Oct-2013	Protocol Amendment 1	01-Oct-2013
08-Oct-2013	Test Substance Administration	08-Oct-2013
15-Nov-2013	Study Records (I-1)	15-Nov-2013
15-Nov-2013	Study Records (Rx-1)	15-Nov-2013
15-Nov-2013, 19-Nov-2013	Draft Report	19-Nov-2013
06-Jan-2014	Final Report	06-Jan-2014

This study was inspected in accordance with the current GLP Regulation, WIL Research's SOPs, and the Sponsor's protocol. A yearly internal facility inspection is conducted by the WIL Research Quality Assurance Department Research. A status report is submitted to management monthly.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments, and WIL Research's SOPs.



Dustin Risner, BA
Quality Assurance Representative

Date

APPENDIX H. COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY ANALYSIS OF FISCHER TROPSCH-SYNTHETIC PARAFFINIC KEROSENE

Fuel	Syntroleum S-8 FT-SPK
POSF	**4734**
Component	**Mass %**
Aromatics	
Alkylbenzenes	
benzene (C06)	<0.01
toluene (C07)	<0.01
C2-benzene (C08)	<0.01
C3-benzene (C09)	0.01
C4-benzene (C10)	0.02
C5-benzene (C11)	0.01
C6-benzene (C12)	<0.01
C7-benzene (C13)	<0.01
C8-benzene (C14)	<0.01
C9+-benzene (C15+)	<0.01
Total Alkylbenzenes	**0.09**
Diaromatics (Naphthalenes, Biphenyl, etc.)	
diaromatic-C10	<0.01
diaromatic-C11	<0.01
diaromatic-C12	<0.01
diaromatic-C13	<0.01
diaromatic-C14+	0.02
Total Alkylnaphthalenes	**0.03**
Cycloaromatics (Indans, Tetralins, etc.)	
cycloaromatic-C09	<0.01
cycloaromatic-C10	<0.01
cycloaromatic-C11	<0.01
cycloaromatic-C12	<0.01
cycloaromatic-C13	<0.01
cycloaromatic-C14	<0.01
cycloaromatics-C15+	0.03
Total Cycloaromatics	**0.05**
Total Aromatics	**0.16**
Paraffins	
iso-Paraffins	
C07 and lower-iso	0.24
C08-isoparaffins	2.31
C09-isoparaffins	6.67
C10-isoparaffins	9.56
C11-isoparaffins	11.10

C12-isoparaffins	11.32
C13-isoparaffins	11.09
C14-isoparaffins	9.62
C15-isoparaffins	7.40
C16-isoparaffins	5.57
C17-isoparaffins	3.28
C18-isoparaffins	1.18
C19-isoparaffins	0.28
C20-isoparaffins	0.08
C21-isoparaffins	0.03
C22-isoparaffins	0.16
C23-isoparaffins	<0.01
C24-isoparaffins	<0.01
Total iso-Paraffins	**79.90**
n-Paraffins	
n-C07 and lower	0.18
n-C08	1.32
n-C09	2.57
n-C10	3.28
n-C11	3.39
n-C12	2.82
n-C13	2.19
n-C14	1.66
n-C15	0.93
n-C16	0.55
n-C17	0.17
n-C18	0.03
n-C19	0.01
n-C20	<0.01
n-C21	<0.01
n-C22	<0.01
n-C23	<0.01
Total n-Paraffins	**19.11**
Cycloparaffins	
Monocycloparaffins	
C07 and lower-monocycloparaffins	0.00
C08-monocycloparaffins	0.19
C09-monocycloparaffins	0.31
C10-monocycloparaffins	0.18
C11-monocycloparaffins	0.07
C12-monocycloparaffins	<0.01
C13-monocycloparaffins	<0.01
C14-monocycloparaffins	<0.01
C15-monocycloparaffins	<0.01
C16-monocycloparaffins	<0.01
C17-monocycloparaffins	<0.01
C18-monocycloparaffins	<0.01

C19+-monocycloparaffins	<0.01
Total Monocycloparaffins	**0.77**
Dicycloparaffins (Decalins, Bihexyls, etc.)	
C08-dicycloparaffins	<0.01
C09-dicycloparaffins	<0.01
C10-dicycloparaffins	0.01
C11-dicycloparaffins	0.01
C12-dicycloparaffins	<0.01
C13-dicycloparaffins	<0.01
C14-dicycloparaffins	<0.01
C15-dicycloparaffins	<0.01
C16-dicycloparaffins	<0.01
C17+-dicycloparaffins	0.01
Total Dicycloparaffins	**0.06**
Tricycloparaffins	
C10-tricycloparaffins	<0.01
C11-tricycloparaffins	<0.01
C12-tricycloparaffins	<0.01
C13-tricycloparaffins	
C14-tricycloparaffins	
C15-tricycloparaffins	
C16-tricycloparaffins	
C17-tricycloparaffins	
Total Tricycloparaffins	**<0.01**
Total Cycloparaffins	**0.82**
Total Aliphatics	**99.84**
TOTAL	**100.00**
Average Molecular Formula - C	11.78
Average Molecular Formula - H	25.53

LIST OF ACRONYMS

AFB	Air Force Base
AFRL	Air Force Research Laboratory
APR	aqueous phase reforming
ARA	Applied Research Associates
AVMA	American Veterinary Medical Association
BMDS	BioMedic Data Systems
CLEEN	Continuous Lower Emissions, Energy and Noise
DLA	Defense Logistics Agency
DoD	Department of Defense
DSHC	direct sugar to hydrocarbon
EPA	Environmental Protection Agency
FAA	Federal Aviation Administration
FT-SPK	Fischer-Tropsch synthetic paraffinic kerosene
GC x GC	Comprehensive two-dimensional gas chromatography
GLP	Good Laboratory Practices
HA JP-8	high aromatic JP-8
HDCJ	hydroprocessed depolymerized cellulosic jet
HEFA-SPK	hydroprocessed esters and fatty acid-synthetic paraffinic kerosene
HJF	Henry M. Jackson Foundation for the Advancement of Military Medicine
HRIPT	human repeat insult patch testing
OECD	Organisation for Economic Cooperation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
PDII	Primary Dermal Irritation Index
Readi	Renewable, Aromatic, Drop-in
SAK	synthetic aromatic kerosene
SOP	standard operating procedure
WIL	WIL Research Laboratories, LLC